Biological chip capable of being disassembled and assembled

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Abstract

The detachable biochip with one solid carrier and capable of being used in analyzing several specimens consists of base plate, probe plate on the base plate, perforated plate on the probe plate with holes corresponding to the array probes, bolt mechanism for positioning the probe array, and irreversible sealing mechanism to form sealing between the probe plate and the perforated plate. The probeplate includes several probe arrays, each of which may be used in several analyses. Before scanning, the sealing mechanism makes the liquid medium in different zones maintained separately, and during scanning, the sealing mechanism is detached for near distance scanning. The present invention has enlarged analysis range, high analysis speed and relatively low biochip analysis cost.

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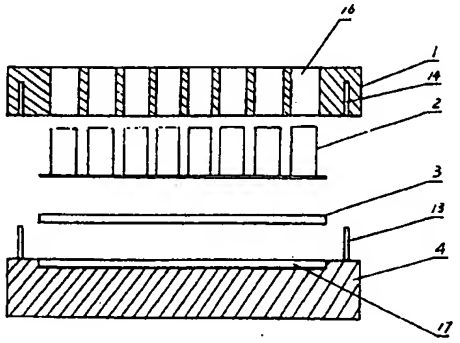
[54] 发明名称 一种可装拆使用的生物芯片

[57] 摘要

本发明公开了一种仅用一个固相载体可对多个样品进行分析的可装拆使用的生物芯片，特征是它由底板、装在其上的探针板、装放在探针板上、板面打有与探针板各探针阵列区位相对应的成孔板，使芯片定位的插销机构，以及使探针板与成孔板之间的接触面形成不泄漏密封的不可逆密封机构所组成，包括多个探针阵列，每个阵列都能进行多项分析的探针板，及可装拆的密封机构；此生物芯片在扫描前，各区位由于此密封机构而使液体介质互不越位，扫描时可拆下隔离密封机构进行近距扫描，其优点是在使用过程中可根据需要进行装拆，既扩大了分析范围又提高了分析速度，同时在保证质量扫描基础上，实现较低成本的生物芯片分析。



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1、一种可装拆使用的生物芯片，其特征在于：它由底板（4），探针板（3），成孔板（1），定位销（13）及非不可逆密封机构组成；底板（4）上装有外表面印刷有M个探针阵列的探针板（3），成孔板（1）上打有与探针板（3）上的探针阵列区域相对应、赋予反应池以高度的M个孔眼（16），成孔板放置在探针板（3）上，通过设置在成孔板（1）与底板（4）上的销孔（14）与销（13）组成的销连接使整个芯片定位，通过非不可逆密封机构使探针板（3）与成孔板（1）之间的接触面形成不泄漏密封；可根据需要在使用时进行装拆，即在需要同一芯片上各探针阵列区位互相隔离时，装配成可互相隔离的结构，在不需要隔离时将隔离结构拆除。

2、按照权利要求1所述的生物芯片，其特征在于：探针板（3）上的探针阵列所在区域及其边界均处于同一平面上（高差小于0.2mm），可有多个独立的探针阵列，处理多个样品。具有这些特征的探针板，只能够在本发明的可装拆生物芯片中使用。

3、按照权利要求1所述的生物芯片，其特征在于：成孔板（1）上有与探针板(3)上的M个探针阵列所在活性区域对应的M个孔眼(16)，这些孔眼有足够的高度，以保证其与上述探针板（3）形成的M个反应池有足够的高度，保证加样、孵育、清洗等与实验有关的反应在各反应池中独立进行。

4、按照权利要求1所述的生物芯片，其特征在于：隔离套（2）是M个由橡胶或塑料制成的具有密封性能的一个腔内中空的柱形套，在需用时将它们放置在探针板（3）上，分别把各探针阵列区位隔离密封起来并嵌入在成孔板（1）的孔眼（16）中，用以将反应液与成孔板相隔离。

5、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封机构是由成孔板（1）在自身重力作用下，紧密地压合在探针板（3）上，形成M个独立互不渗透的反应池所构成的机构。

6、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封机构是由成孔板（1）在真空管（5）所抽真空的作用下，紧密地吸附在探针板（3）上，形成M个独立互不渗透的反应池所构成的机构。

7、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封

机构是由成孔板（1）在螺钉（7）拧紧力作用下，紧密地压合在芯片（3）板上，形成M个独立互不渗透的反应池所构成的机构。

8、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封机构是由成孔板（1）在外界压力（8）作用于下，紧密地压合在探针板（3）上，形成M个独立互不渗透的反应池所构成的机构。

9、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封机构是由成孔板（1）在卡头（9）和卡槽（10）之间的卡紧力作用下，紧密地压合在探针板（3）上，形成M个独立互不渗透的反应池所构成的机构。

10、按照权利要求1所述的生物芯片，其特征在于：其非不可逆密封机构是由成孔板（1）在磁铁[永磁铁（11）或电磁铁（12）]的磁力作用下，紧密地压合在探针板（3）上，形成M个独立互不渗透的反应池所构成的机构。

一种可装拆使用的生物芯片

技术领域

本发明涉及一种生物芯片，特别是一种多阵列生物芯片。

背景技术

生物芯片，是指含有包被在固相载体上的具有生物活性物质的微点阵（包括 DNA，抗原，抗体，细胞，药物等生物活性微点阵），并可以对其进行孵育，洗涤等亲和反应和纯化操作，可以通过扫描仪等装置对标记物进行识别的一种分析产品。按照包被在固相材料上的具有生物活性的亲和物质的探针阵列数目的不同，生物芯片可分为单阵列芯片和多阵列芯片。单阵列芯片在设计上只能对一分样品进行分析，多阵列芯片在设计上能够对一分以上的M分样品进行分析。按照芯片表面结构的不同，生物芯片有平面芯片及凹面芯片。目前，平面芯片只用于单阵列芯片，凹面芯片主要用于多阵列芯片。平面芯片仅有一个平面，探针阵列所在区域及其边界面处于同一平面。其生产方法为，直接将生物探针固定于此一平面板上形成探针阵列，无需再作成型处理即可使用。凹面芯片有二个以上平面，其探针阵列所在区域低于该区域的边界面，从而形成一反应池。所有的加样，孵育，洗涤等操作均在反应池内进行。现有的凹面生物芯片的生产方法有两种。第一种方法是先成型后固定探针，即通过模压，磨孔等工艺在芯片材料上形成凹面，然后将生物探针固定于每一已成型的凹面上形成一个独立的探针阵列。第二种方法为先固定探针后成型，即将生物探针首先固定于一平板上形成M个探针阵列，然后再用粘合或嵌合等工艺制成一个复合凹面，以作反应池用。现有的凹面芯片的生产方法，只能生产带永久性凹面的芯片。平面芯片的优点在于对扫描仪的限制少，可对标记物进行近乎零距离的扫描，缺点是只有一个探针阵列，只能处理一个样品。而现在的凹面芯片虽可包被多个探针阵列且每个探针阵列均处于一个凹型反应池中，易于加样，孵育，洗涤等反应的操作，但缺点是在扫描时，由于具有永久性反应池高度及凸出的界面，比之平面芯片对扫描仪要有较多的特殊要求，因而大大地限制了其应用。

发明内容

本发明的目的旨在克服上述现有芯片的缺点，为用户提供一种在反应时具有凹面结构，而在扫描时具有平面结构的可装拆使用的生物芯片。这种芯片在完成反应需要足够高的反应池高度时，用两层或两层以上的材料，经接触面的非不可逆密封而成；在进行扫描不需要反应池高度时，仅需将含有探针阵列的探针板与提供反应池高度的其它材料，以物理方式分离开来即可。

本发明的目的是这样实现的：

一种可装拆使用的生物芯片，其特征在于可根据需要在使用时进行装拆，即在需要同一芯片上各探针阵列互相隔离时，装配成可互相隔离的结构，在不需要隔离时将隔离结构拆除。这种芯片的核心为一个固定有M个探针阵列的平板（探针板），这种芯片的基本结构由探针板、底板、隔离套（需要时），成孔板，定位销及非不可逆密封机构组成（基本结构如图1所示）；底板上放置外表面印刷有M个探针阵列的探针板，探针板的板面上放置成孔板，成孔板上打有与探针板上M个探针阵列相对应的赋予反应池以高度的M个孔眼，（在需要的时候，也可将含有M个隔离空心柱的一次性使用的隔离套，嵌入成孔板的M个孔眼中，将反应液相介质与成孔板相隔离），放置在探针板的成孔板上，通过设置在其板面上与底板上的定位销孔与销组成的销连接使整个芯片定位，通过非不可逆密封机构使探针板与成孔板之间或探针板、隔离膜、成孔板之间（在使用隔离膜的情况下）的接触面形成不泄漏密封；

这种可装拆使用的生物芯片，其生产方法亦与目前生物芯片的生产方法大不相同：1）它可以分部件独立生产，仅在使用时再组合安装，2）可直接将生物探针固定在平面基片上形成M个阵列，不再进行其它形成永久性凹凸结构的工艺处理，即可与其它部件直接使用，3）除探针板外，所生产的其它部件可以是一次性使用的，也可以是反复使用的。

本发明中的探针板，其特征为：一个平板上有M（$M \geqslant 1$）个独立的探针阵列，探针阵列所在区域及其边界均处于同一平面上（高差小于0.2mm），每一个探针阵列，在使用中可处理一个样品。该探针板的材料可以是玻璃，塑料，胶片及其它可用于生物芯片基片制作的材料。本发明中探针板的生产，与目前生物芯片的生产可以完全区别开来：由于它在一个板上可固定多个探针阵列而同现有的平面芯片生产区别开来，由于它是在一个平板上固定多个探针阵列后再无其它生成凹面的生产工

艺加工，就可直接供给用户而同现有的凹面芯片区别开来。

本发明中的成孔板，其特征为：板上有与上述探针板上M个探针阵列所在局部区域对应的M个孔，这些孔有足够的高度，以保证其与上述探针板区域形成的M个反应池有足够的高度，能保证加样、孵育、清洗等与实验有关的反应在各反应池中独立进行；该成孔板可以是一种材料，如含铁或不含铁的塑料，玻璃，陶瓷，不锈钢，也可以是由这些材料复合而成，例如在不锈钢的底面加一层橡胶膜以增加密封性能及保护探针板。成孔板可以是一次性使用的，也可以是多次使用的。其生产，可以与探针板配套进行，也可以独立进行，还可以作为生物芯片设备的一个部件来生产。

本发明的隔离套，其特征为：含有M个由橡胶或塑料或橡胶/塑料复合物（例如塑料空心柱加橡胶垫膜）制成的腔内中空的柱形套，在需用时将它们放置在探针板上，分别把各探针阵列区位隔离密封起来并嵌入成孔板的孔眼中，用以将反应液与成孔板隔离开，确保成孔板在使用过程中不会污染而可重复使用。本发明的隔离套，为一次性使用部件。其生产，可以与探针板配套进行，也可以独立进行，还可以作为生物芯片设备的一个部件来生产。

本发明中的非不可逆密封机构，其特征为：1）在探针板与成孔板之间的所有接触面（在不使用隔离套时仅为成孔板/探针板一个接触面，使用隔离套时有成孔板/隔离套/探针板二个接触面）上形成密封，避免反应介质透过此接触面泄漏，出现反应池间的污染；2）上述密封是非不可逆的，即在需要反应池有足够高度时，上述接触面必须处于密封状态，而在不需要反应池高度时，上述接触面是可以分离的。此一密封机构的具体形式包括有：①成孔板在自身重力作用下足够紧密地压合在探针板上，形成M个独立互不渗透的反应池；②成孔板上的橡胶凹面在抽真空作用下紧密地附着在探针板上，形成M个独立互不渗透的反应池；③成孔板在螺钉拧紧力作用下紧密地压合在探针板上，形成M个独立互不渗透的反应池；④成孔板在外界压力作用于下紧密地压合在探针板上，形成M个独立互不渗透的反应池；⑤成孔板在卡头和卡槽之间的卡紧力作用下紧密地卡合在探针板上，形成M个独立互不渗透的反应池；⑥成孔板在磁铁（永磁铁或电磁铁）磁力作用下紧密地压合在探针板上形成M个独立互不渗透的反应池。

本发明中的底板，是上述非不可逆密封机构及对位装置的一个部件，其特征为：是非不可逆密封机构中不可缺的部分及固定有对位装置，其制作材料可以是含铁或不含铁的塑料，橡胶，陶瓷，玻璃，钢材，磁铁等材料及它们的复合物，例如在钢材表面附一层橡胶以保护探针板。其生产，可以与探针板配套进行，也可以独立进行，还可以作为生物芯片设备的一个部件来生产。

本发明的优点在于：以极小的生产成本，实现了生物芯片在反应时（包括加样、孵育、洗涤等操作）具有较高自由度的反应条件（例如：反应池高度，体积等），在扫描时具有较高自由度的扫描条件（例如：整个芯片在扫描时处于同一平面，整个芯片的高度可最小化），从而以极小的成本实现了较高自由度多阵列生物芯片的生产，为在同一探针板上进行多样品的多种分析，提供一个经济可行的方案。

附图说明

图 1 为本发明芯片结构纵剖示意图

图 2 为本发明探针板示意图

图 3 为本发明依靠成孔板重力实现非不可逆密封例子的芯片结构纵剖示意图

图 4 为本发明依靠成孔板与探针板接触面处抽真空实现非不可逆密封例子的芯片结构纵剖示意图

图 5 为本发明依靠螺纹连接力实现非不可逆密封例子的剖芯片结构纵剖示意图

图 6 为本发明依靠外界机械压力实现非不可逆密封例子的芯片结构纵剖示意图

图 7 为本发明依靠卡式联接实现非不可逆密封例子的芯片结构纵剖示意图

图 8 为本发明依靠永磁铁吸力实现非不可逆密封例子的芯片结构纵剖示意图

图 9 为本发明依靠电磁铁吸力实现非不可逆密封例子的芯片结构纵剖示意图

图中标记：1 为成孔板，2 为隔离套，3 为探针板，4 为底板，5 为抽真空管路，6 为螺纹孔，7 为螺钉，8 为外力，9 为卡头，10 为卡槽，11 为永磁铁，12 为电磁铁，13 为定位销，14 为定位销孔，15 为成孔板自

身配重，16 为成孔板上的孔眼，17 为探针板安装槽。

具体实施方式

下面结合实施例附图，对本发明作进一步详细说明:

本发明可装拆使用的生物芯片的基本结构如图 1 所示，它由成孔板 1，探针板 3，底板 4，定位销 13 组成；探针板 3 放置于底板 4 的凹槽 17 内，在需隔离时隔离套 2 嵌入成孔板 1 的孔眼 16 中，并位于成孔板 1 与探针板 3 之间，定位销 13 将整个生物芯片定位。在物理作用下实现密封作用，成孔板 1 上的孔眼与探针板 3 之间（在有隔离套的情况下，为隔离套的柱孔与探针板 3 之间）形成 M 个密封的反应池。

例 1: 多阵列探针板的生产

利用现在的点样机（本实验使用美国 Microarray printer XMM47.832 型手动点样机），丙肝抗原（北京儿童医院肝病研究所），丙肝肽抗原 P21（法国 SEDAC 公司），丙肝 NS4 区肽抗原（法国 SEDAC 公司）及丙肝 NS3 区蛋白质抗原（北京儿童医院肝病研究所），共四种探针，以 1mg/me 的浓度，点到一（宽 x 长 x 厚）尺寸为 25x75x1mm 的已经作了活化处理的玻片上（法国 SEDAC），其点样方式为: 每一种抗原有四个探针，四种抗原的十六个探针形成一个 4x4 阵列，每一个探针阵列的尺寸为 4x4mm，每一玻片上有 2 列 14 个探针阵列，每列有 7 个。点样后用封闭液封闭，然后清洗，吹干，即制成备用的 14 个阵列的 4 抗原探针板（图 2）。

例 2: 由重力进行非不可逆密封的例子（图 3）

在本发明中，成孔板 1 在自身的重力作用下，紧密地压在探针板 3 上，依靠成孔板 1 自身的重力，配合隔离套 2 的作用，实现成孔板 1 与探针板 3 之间形成 M 个密封的反应池。在吹干后不需要反应池时，仅需将嵌入有隔离套的重力成孔板撤去，便可获得供扫描的多探针阵列平板芯片。

例 3: 通过抽真空实现非不可逆密封的例子

如图 4 所示，成孔板 1 上有真空管路 5，当装了隔离套 2 的成孔板 1 压在探针板 3 上时，通过抽空管路 5 向外抽真空，在大气压力作用下将成孔板 1、隔离套 2、探针板 3 三者紧密地压在一起，形成 M 个密封的反应池。不需要反应池时，仅需打开抽真空管路使其充上空气，即可撤去隔离套及成孔板，只用探针板。

例 4：通过螺纹结构实现非不可逆密封的例子

如图 5 所示，成孔板 1 和底板 4 上有相对应的螺纹孔 6，使用螺钉 7 将成孔板 1、隔离套 2、探针板 3、底板 4 紧密地结合在一起，形成 M 个密封的反应池，不需要反应池时，仅需扭开螺打 7，即可撤去成孔板隔离套，留用探针板。

例 5：通过外部机械重力实现非不可逆密封的例子

如图 6 所示，成孔板 1、隔离套 2、探针板 3、底板 4 在外力 8（如外加压力，钳力等）作用下，紧密地压合在一起，形成 M 个密封的反应池。不需要反应池时，仅需撤除外力将螺纹扭转，即可卸下成孔板及隔离套，取出探针板。

例 6：通过卡式连接实现非不可逆密封的例子

如图 7 所示，成孔板 1 上有两个卡头 9，底板 4 上有两个卡槽 10，将探针板 3 放在底板 4 上，并将装了隔离套 2 的成孔板 1 放在探针板 3 上后，将两个卡头 9 压入卡槽 10 中，实现成孔板 1、隔离套 2、探针板 3、底板 4 之间紧密结合，形成 M 个密封的反应池。不需要反应池时，仅需下压卡头9解除与卡槽10间的卡扣联接，即可卸下成孔板及隔离套，取出探针板。

例 7：通过永久磁铁实现非不可逆密封的例子

如图 8 所示，在底板 4 中装有永久磁铁 11，成孔板 1 的材料是不锈钢（在不使用隔离套时为不锈钢和一层橡胶的复合材料），当将探针板 3、隔离套 2、成孔板 1 依次放在底板 4 上时，磁铁 11 对钢质成孔板 1 有较大的吸力，在此吸力作用下实现成孔板 1、隔离套 2、探针板 3、底板 4 之间紧密结合，形成 M 个密封的反应池。

例 8：通过电磁铁实现非不可逆密封的例子

如图 9 所示，在底板 4 中装有电磁铁 12，成孔板 1 的材料是不锈钢（在不使用隔离套时为不锈钢和一层橡胶的复合材料），将探针板 3、隔离套 2、成孔板 1 依次放在底板 4 上，当电磁铁 12 通电，对钢质成孔板 1 有较大的吸力，在此吸力作用下实现成孔板 1、隔离套 2、探针板 3、底板 4 之间紧密结合，形成 M 个密封的反应池。

例 9：反应与扫描的例子

通过例 1 制作的探针板，然后通过例 2 至例 8 获得的 7 个不同的装置芯片，在本例中经过亲和反应，纯化反应后，拆开隔离组件将带有标

记物的探针板进行了扫描。其过程完全一样:

从丙肝标准品（购自中国生物制品与药检定所）中选择3个阴性样品（编号1^-, 2^-, 21^-）及9个阳性样品（编号1^+, 4^+, 7^+, 21^+, 24^+, 31^+, 35^+, 38^+, 39^+, ），用封闭液对这些样品作20倍稀释，然后各取50ul稀释样品分别加到此7个芯片的反应池中。在37^0C孵育1小时，再分别用50ul洗涤液洗涤四次。再分别取50ul用封闭液稀释的罗丹明标记IgG-A-M加入芯片的反应池中，37^0C下孵育1小时. 孵育出来后，用洗涤液对每个反应池洗涤四次，蒸馏水洗涤二次，无水乙醇洗片一次，室温晾干。至此反应已结束，然后根据芯片不同的非不可逆密封机构，拆出隔离密封组件取出探针板，分别放入扫描仪进行扫描及数据处理，所得结果如下:

表1（例4所获）：生物芯片在例8试验中的结果

血清样品	RIBA结果	生物芯片分析结果			
		Anti-HCV	Core	NS3	NS4
1^-		545(45)			
2^-		657(63)			
21^-		589(59)			
1^+	$NS3^+$	13254(768)	325(59)	352(49)	348(52)
4^+	$NS3^{++}$	7453(879)	469(56)	5498(420)	505(42)
7^+	$NS3^{++++}$	12432(650)	342(54)	4563(654)	324(29)
21^+	$NS3^{++++}$	28547(3965)	356(41)	13568(2904)	367(41)
24^+	$NS3^{+++}$+$NS4^+$	21784(2509)	395(42)	481(65)	7548(1241)
31^+	$NS3^+$+$NS4^-$	12461(1973)	387(54)	5248(657)	21765(1534)
35^+	$NS3^{++++}$	25423(1795)	476(67)	6830(597)	501(67)
38^+	$NS3^+$	10568(798)	524(45)	8675(805)	596(60)
39^+	$Core^{++}$	39034(2543)	43985(3098)	289(45)	286(67)
空白试验	5833(49)				

以上数据均为统计平均数值，括号中的数值为标准差。

7种不同芯片所获得的结果一致（结果略），这些结果同用目前标准的平面芯片所获得的结果（结果略）也是一致的。

本发明涉及的生物芯片，既能在反应时防止反应池之间的互相渗漏，又能在扫描时使芯片只有一个平面，从而大大减小了对M个样品进行分析时的技术难度，并使分析成本大大降低。

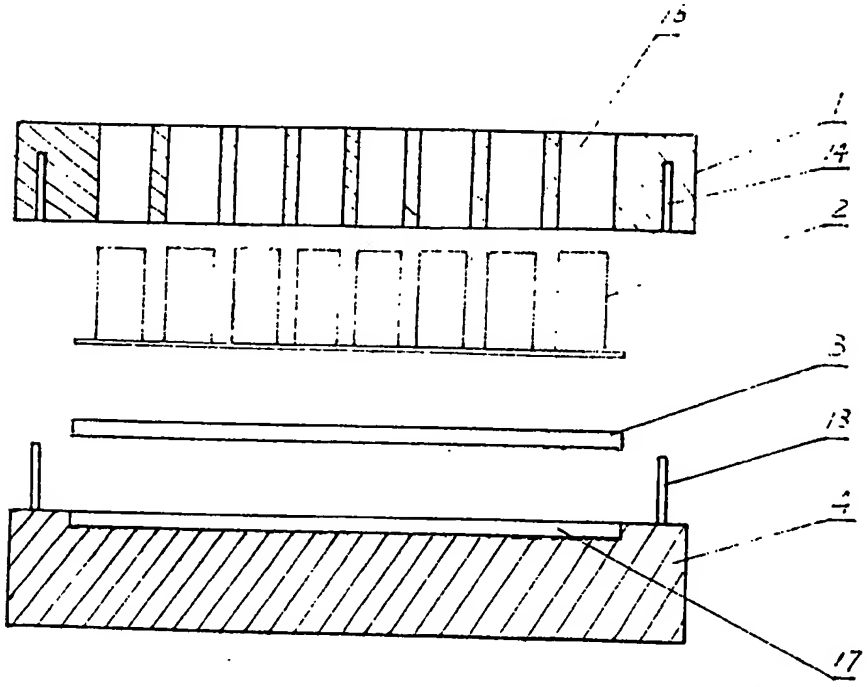

图 1

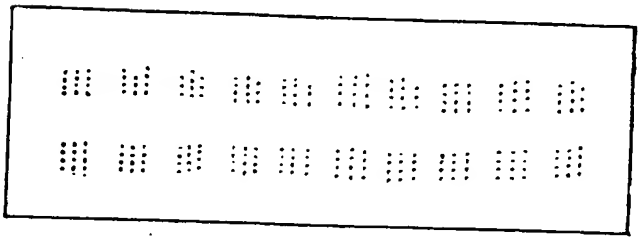

图 2

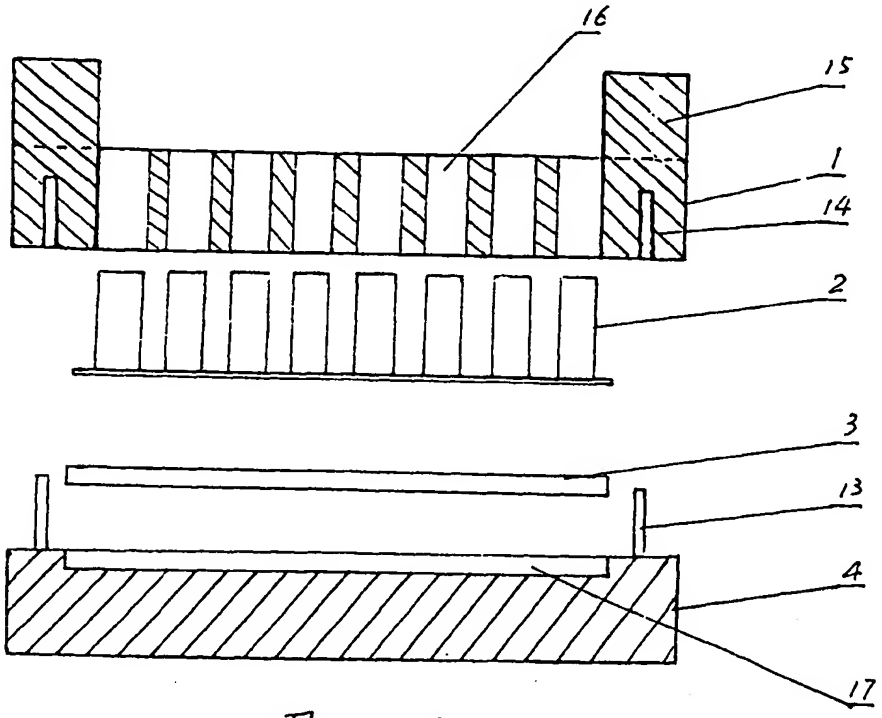

图 3

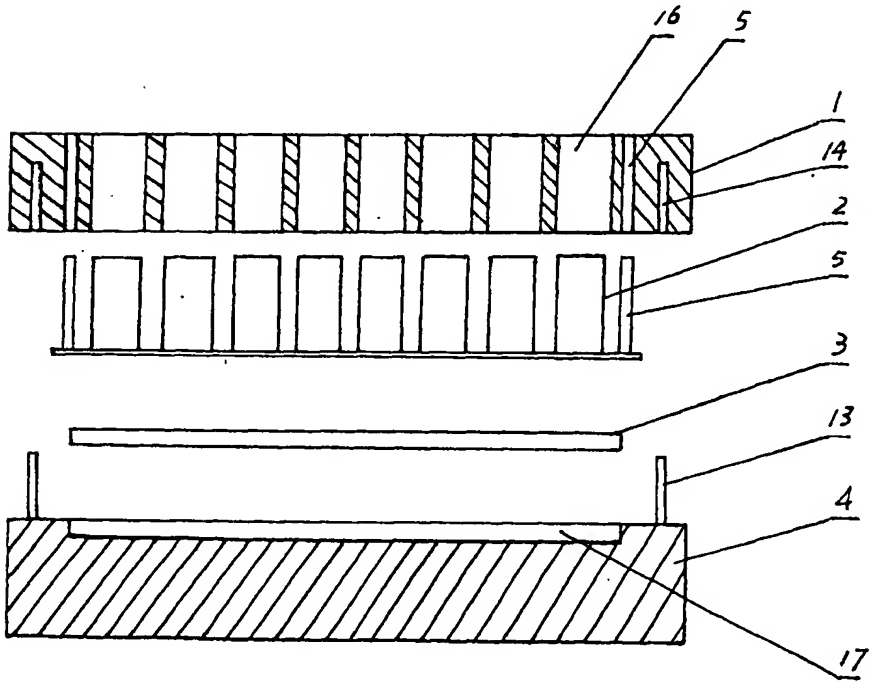

图 4

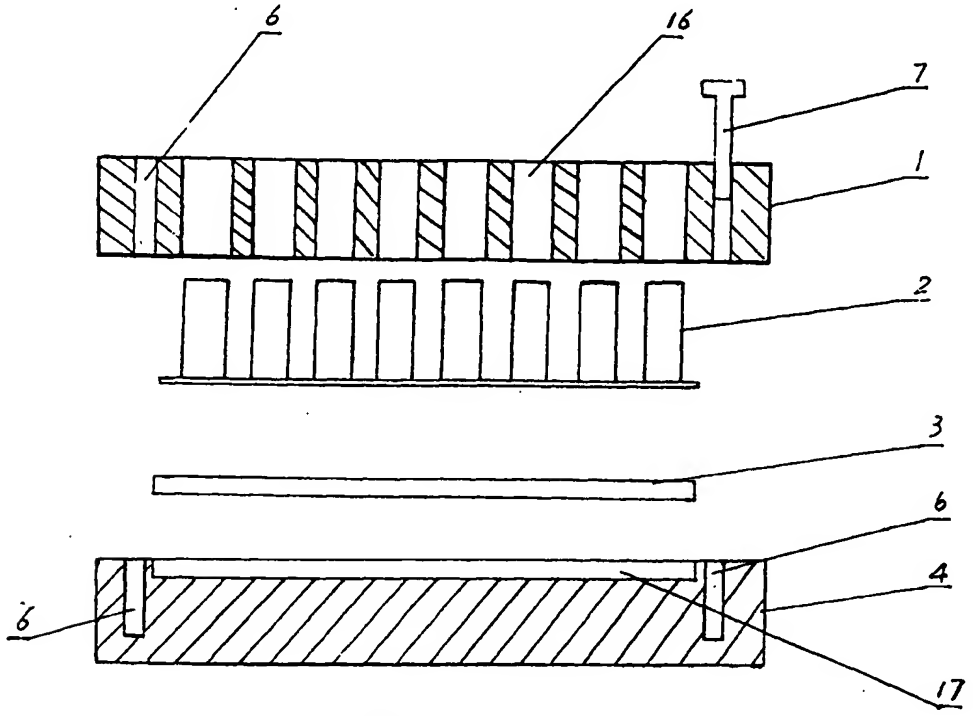

图 5

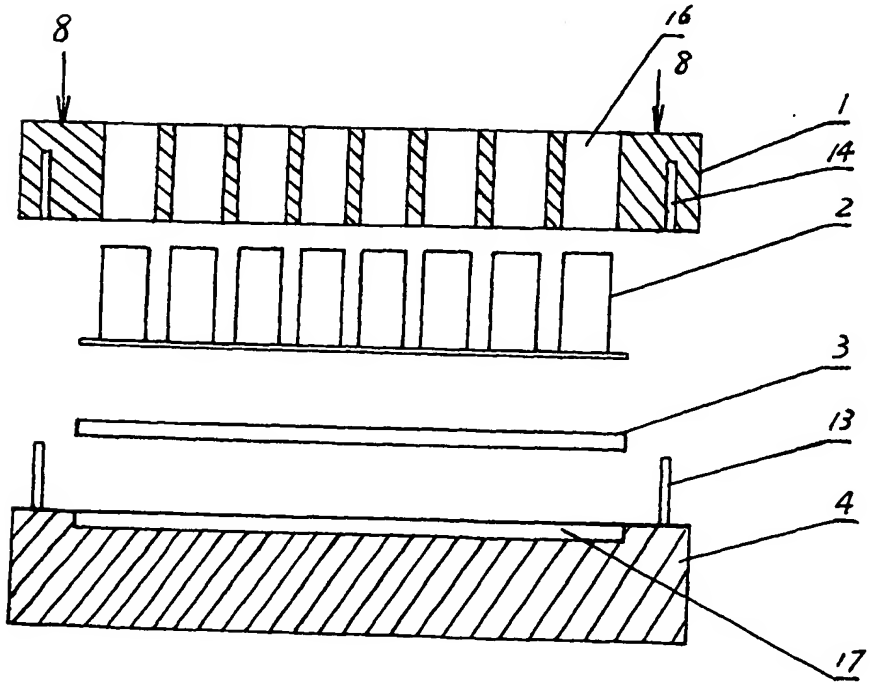

图 6

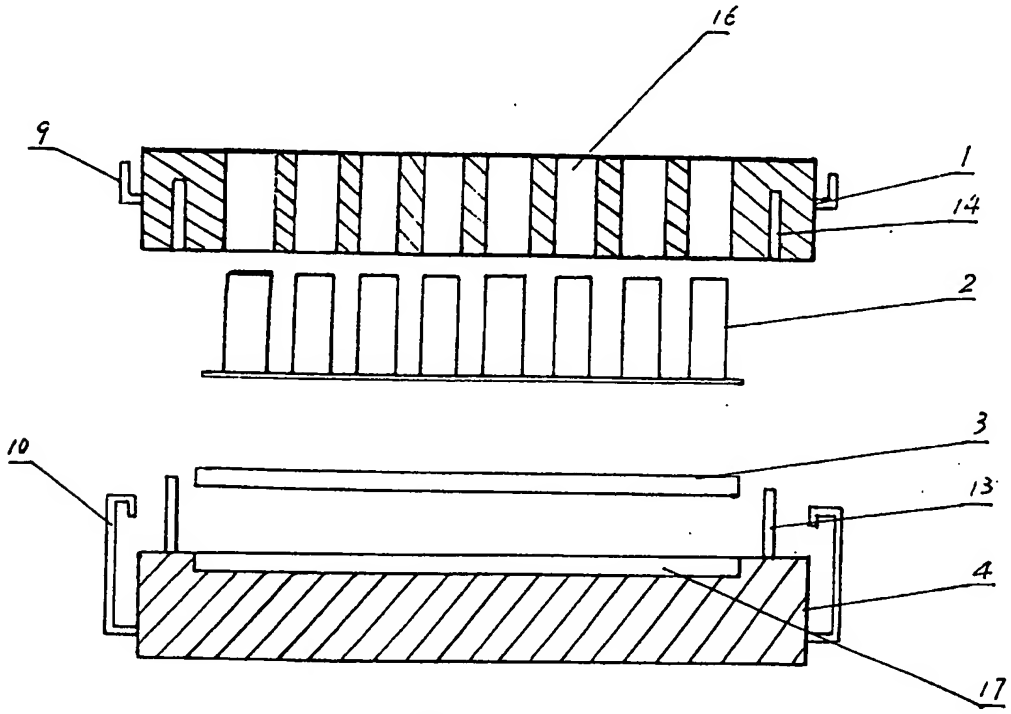

图 7

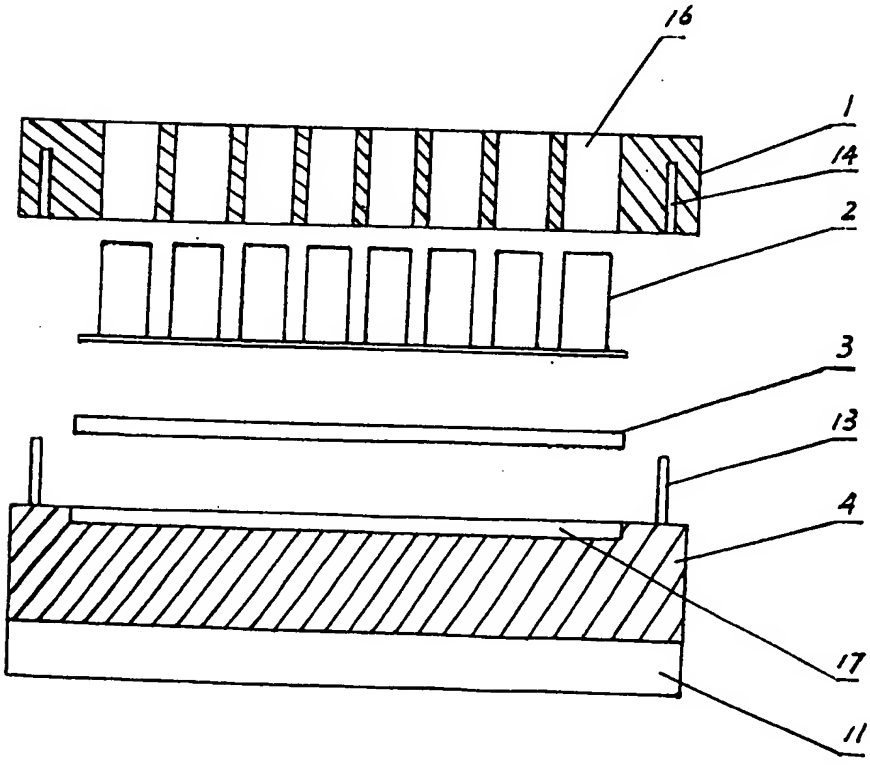

图 8

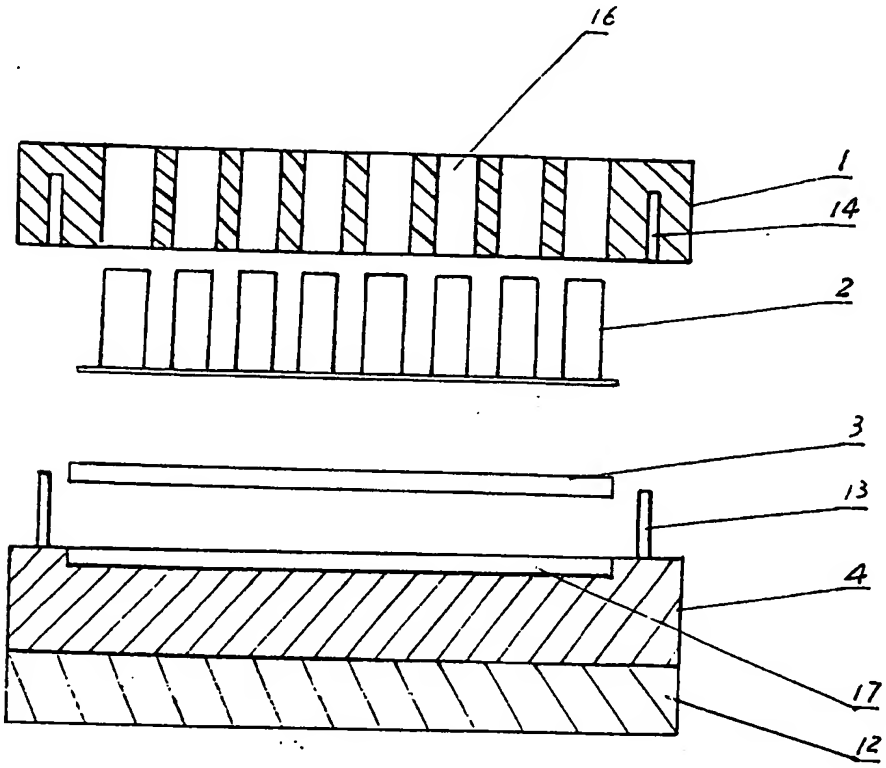

图 9

(Chinese patent application number 02113540.1)

Claims

What is claimed is:

1. A kind of biochip can be installed and dismantled, it comprises of a setting(4), a probe-plate(3), a hole-plate(1), localizing pins(13) and reversible sealing structure; the probe-plate with M probe arrays is installed on the setting, the hole-plate has M holes(16), which correspond to the probe arrays on the probe-plate and form the height of the reactor wells, the hole-plate is placed on the probe-plate, and the biochip is forelocked by the pin hole and the pins on the hole-plate and the setting, the contact surfaces of the hole-plate and the setting form un-leakage seal through reversible sealing structure.

2. The biochip of Claim 1, it has several independent said probe arrays and can test multi-samples, the region of probe arrays on the probe-plate and its outskirts are on the same plane(the height difference < 0.2mm).

3. The biochip of Claim 1, the hole-plate has M holes correspond to the M probe arrays on the probe-plate, the holes have enough height to ensure forming the height of the reactor wells enough for the relative test steps carrying out independently in each well, such as the sample subjecting, incubation, wash etc.

4. The biochip of Claim 1, its separate sheath(2) has M hollow columniation sheathes made of rubber or plastic and are of sealing characteristics. When the separate sheath was used, it was put on the probe-plate(3), each of the probe array region is hermetically closed, and the probe array region is set in the hole(16) of the hole-plate(1) and the reaction reagent and the hole-plate are separated.

5. The biochip of Claim 1, its reversible sealing structure is structured by the gravity weight of the hole-plate itself to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

6. The biochip of Claim 1, its reversible sealing structure is achieved by the hole-plate vacuumed by vacuum pipe to impact onto the probe-plate to form M independent and un-leakage sealing reactive wells.

7. The biochip of Claim 1, its reversible sealing structure is achieved by the hole-plate under the force of screws(7) to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

8. The biochip of Claim 1, its reversible sealing structure is achieved by the hole-plate under the outside force(8) to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

9. The biochip of Claim 1, its reversible sealing structure is achieved by the hole-plate under the force of positive fasteners（9）and negative fasteners(10) to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

10. The biochip of Claim 1, its reversible sealing structure is achieved by the hole-plate under the force of magnet（permanent magnet (11) or electromagnet(12)）to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

A kind of biochip can be installed and dismantled

Technical scope

This invention involves a kind of biochip, especially a kind of multi-probe-array biochip.

Background techniques

Biochip is an analysis device including microarray of biological activity material(DNA, antigen, antibody, well, medication, etc.) coated on the solid support, can be incubated, wash affinity reaction and purification operations, and the marker can be identified through array scanner. According to the different amount of microarray of biological activity material coated on the solid support, the biochip can be divided into mono-array biochip and multi-array biochip. The mono-array biochip is designed for assay of one sample, the multi-array biochip is designed for assay of more than one sample. According to the different surface structure, the biochip can be divided into plane biochip and concave biochip. The plane biochip is used for one array biochip, the concave biochip is used for multi-array biochip.The probe-array region and its outskirts of the plane biochip is on one plane, its manufacturing method is to immobilizing bio-probe on the plane and form the probe array, it need not molding and can be used. The probe-array region and its outskirts of the concave biochip is on two or more planes, its probe array region is lower than the plane of the outskirts region and forms a reactive well. All the operations of sample subjecting,

incubation and washing are in the reactive well. The manufacturing methods of concave biochip have two kinds. One of the methods is molding the solid support before the probe immobilized, namely the concave is made through the process of mould pressing or hole grinding, etc, then the probes are immobilized on each the concave to form an independent probe array. The second method is the probe immobilized before molding the solid support, namely the probes are immobilized to form M probe arrays, then the concaves are made by the process of conglutination or embed to act as reactive wells. The manufacturing methods of concave biochip can only produce permanent concave biochip.

The advantages of the plane biochip are little of the limit to the biochip scanner and the labeled target molecules can be scanned nearly at zero distance, the disadvantage is that has only one probe array, can only test one sample. The prevalent concave biochip has multi-probe-array coated and each of the probe arrays is in a concave reactive well, the advantages are easy for the process of sample subjecting, incubation and washing, etc. The disadvantage is because of the permanent height and the protrude outskirts of the reactive well, the concave biochip has more special scanning conditions than that of plane biochip, thus the usage of concave biochip is limited greatly.

Invention contents

The aims of this invention are to overcome the disadvantages said above and provide a kind of reversible connection biochip, it has the concave structure during its reaction and has the plane structure when its scanning. When the reversible connection biochip need enough height of reactive well for reaction, the two or more layers are used, and the reactive well are formed by the contact surfaces of the layers reversible seal. When the reversible connection biochip need not height of reactive well for scanning, the probe-plate and the layers providing the height of reactive well are divided by physical manner.

A kind of biochip using reversible connection parts, the characteristic is: it can be assembled and detached during its using, namely when the probe arrays on the biochip need isolation each other, it can be assembled to form partition structure, and the partition structure can also be detached when need not isolation.

The core of the biochip is a plate (probe-plate) immobilized M probe arrays, the basic structures of the biochip are a probe-plate, a setting, partition sheath（when needed）, the hole-plate, localizing pins and reversible sealing structure(see Figure 1);

the probe-plate with M probe arrays is installed on the setting and the hole-plate is placed on the probe-plate, the hole-plate has M holes forming the height of the reactive well and corresponding to the probe arrays on the probe-plate(the partition sheath with M partition hollow columniations for single use may be embed into the M holes on the hole-plate, to separate the reactive reagent and the hole-plate when needed), the biochip is forelocked by the and the pins on the hole-plate and the setting, the contact surfaces of the hole-plate and the setting, or the hole-plate, partition sheath(when the partition sheath is needed) and the setting form un-leakage seal through reversible sealing structure.

The productive method of the biochip using reversible connection parts is different from that of present biochip: 1) each of the parts of the biochip can be produced independently, and assembled when it is employed, 2) the probe arrays can be immobilized directly on the plane support to form M array and can be used with the other parts, without doing the process of forming the permanent structures of concave and protrusion, 3) all the parts except the probe plate may be the one-time use or multi-time use.

The biochip of this invention, it has M($M \geqslant 1$) independent probe arrays and can test multi-samples, the region of probe arrays on the probe-plate and its outskirts are on the same plane(the height difference < 0.2mm), each of the arrays can test a sample. The material of the probe plate can be glass, plastic, film and the other material used for biochip plate. The productive method of the biochip is different from that of present biochip: it is different from present plane biochip because of multi-probe-array can be immobilized on one plane support, it is different from present concave biochip because this invention biochip can be marketed after immobilizing multi-probe-array without doing the process of forming the concave structure.

The biochip of this invention, the hole-plate has M holes correspond to the M probe arrays on the probe-plate, the holes have enough height to ensure forming the height of the reactor wells enough for the relative test steps carrying out independently in each well, such as the sample subjecting, incubation, wash etc. The hole-plate can be made of one or the combination of following materials: plastic, glass, ceramic and stainless steel containing iron or no, for example, the bottom surface of stainless steel is fixed a layer of rubber to increasing the seal characteristics and protecting the probe plate. The hole-plate can be used one time or multi-time, it can be produced together with probe plate, or produced independently, or produced as

a part of the test equipment of biochip.

The separate sheath of this invention has M hollow columniation sheathes made of rubber or plastic or their combination(e.g. plastic hollow columniation and rubber underlay). When the separate sheath was used, it was put on the probe-plate, each of the probe array region is hermetically closed, the probe array region is set in the hole of the hole-plate, the reaction reagent and the hole-plate are separated and insure the hole-plate is not contaminated when using. The separate sheath of this invention is s part for one time use, it can be produced together with probe plate as a mate, or produced independently, or produced as a part of the test equipment of biochip.

The characteristics of reversible sealing structure of this invention are: 1) it can seal up all the surfaces between the probe plate and the hole-plate to avoid the leakage of the reagent through the surface, and avoid the contamination bringing among the reactive wells. 2) the sealing is reversible, the contact surfaces must be sealed when the enough height of reactive well is needed, the contact surfaces can be separated when the enough height of reactive well is not needed.

The formations of sealing structure are: ① the gravity weight of the hole-plate itself to impact onto the probe-plate to form M independent and un-leakage seal reactive wells. ② the concave surface of the hole-plate is vacuumed to impact tightly onto the probe-plate to form M independent and un-leakage seal reactive wells. ③ the hole-plate is under the force of screws to impact tightly onto the probe-plate to form M independent and un-leakage seal reactive wells. ④ the hole-plate is under the pressure of outside to impact tightly onto the probe-plate to form M independent and un-leakage seal reactive wells. ⑤ the hole-plate is under the force of positive fasteners (9) and negative fasteners to impact onto the probe-plate to form M independent and un-leakage seal reactive wells. ⑥ the hole-plate is under the force of magnet (permanent magnet or electromagnet) to impact onto the probe-plate to form M independent and un-leakage seal reactive wells.

The setting of this invention is a integrant part of said reversible sealing and localizing structure, it is a integrant part for sealing structure and localizing structure is fixed on it. It is made of one or the combination of following materials: plastic, rubber, ceramic, glass, steel and magnet containing iron or no, for example, the bottom surface of the steel is fixed a layer of rubber to protect the probe plate. The setting can be produced together with probe plate, or produced independently, or produced as a part of the test equipment of biochip.

The advantages of this invention are: the productive cost of the biochip is low; high

freeness of the reactive condition(the height of reactive well) is achieved; high freeness of the scanning condition(the height of reactive well is nearly zero when scanning) is achieved when the biochip is scanned; multi-sample can be tested on one probe plate, so a economical method of biochip production is provided .

Figure Illustration

Figure 1 is the cutaway sketch map of the structure of the biochip of this invention
Figure 2 is the sketch map of the probe plate of this invention
Figure 3 is the cutaway sketch map of the structure of reversible sealing by gravity weight of the hole-plate of the biochip of this invention

Figure 4 is the cutaway sketch map of the structure of reversible sealing by vacuuming the contact surface of hole-plate and the probe-plate of the biochip of this invention

Figure 5 is the cutaway sketch map of the structure of reversible sealing by the force of screws the biochip of this invention

Figure 6 is the cutaway sketch map of the structure of reversible sealing by the outside machine force of the biochip of this invention

Figure 7 is the cutaway sketch map of the structure of reversible sealing by positive fasteners and negative fasteners of the biochip of this invention

Figure 8 is the cutaway sketch map of the structure of reversible sealing by permanent magnet of the biochip of this invention

Figure 9 is the cutaway sketch map of the structure of reversible sealing by electromagnet of the biochip of this invention

1 hole-plate, 2 eparate sheath, 3 probe plate, 4 setting, 5 vacuuming pipe, 6 screw nut, 7 screw, 8 out side force, 9 positive fasteners, 10 negative fasteners , 11 permanent magnet, 12 electromagnet, 13 localizing pin, 14 localizing hole, 15 hole-plate gravity, 16 the hole of a hole-plate, 17slot for probe plate fixing。

Detailed implementation

Following will give more detailed description with the Figures:

The basic configuration of biochip using reversible connection parts of this invention as Figure 1, it comprises of a hole-plate(1), a probe-plate(3), a setting(4), localizing pins(13). The probe-plate is put into the concave slot(17) of the setting, separate sheath(2) is embed in the holes(16) of the hole-plate when the separation is needed. Sealing function is achieved by physical action, the M sealing reactive wells

are formed by the holes of the hole-plate and the probe plate(when the separate sheath is used, the reactive wells are formed by the columniation holes of the separate sheath and the probe plate).

The production of multi-array probe plate

The probes used are NS4 antigen of HCV, P21 antigen of HCV, NS4 antigen of HCV and NS3 antigen of HCV, the manual array printer used is Microarray printer XMM47.832. The probes of 1mg/ml are printed on a 25×75×1mm activation glass slide (SEDAC, France), each of the probe is spotted 4 spots, 4 probes are 16 spots altogether to form a 4×4 array, the size of a array is 4×4mm. There are 14 probe spots by 2 rows, 7 probe spots each row. The probe plate(Figure 2) is blocked using block reagent after spotting, then washed and dried.

reversible sealing by gravity(Figure 3)

In this implement, the hole-plate(1) with the gravity weight of itself and the action of separate sheath(2) impacts onto the probe-plate(3) to form M sealing reactive wells between the hole-plate(1) and the probe-plate(3). When the biochip is dried and need not the reactive wells, the multi-array plane biochip is achieved by dismantle of the separate sheath and the hole-plate.

Reversible sealing structure is achieved by vacuum

The suction pipe(5) is on the hole-plate(1) as the Figure 4, when the hole-plate installed separate sheath(2) is press on the probe plate(3) and vacuumed through suction pipe, the hole-plate(1), separate sheath(2) and the probe plate(3) are tightly suppressed together by atmosphere pressure to form M sealing reactive wells. When the reactive wells are not needed, the suction pipe is open and the air is in, then the hole-plate and the separate sheath are dismantled, the probe plate can be used for next step of operation.

Reversible sealing structure is achieved by screws

The hole-plate(1) and setting(4) have the corresponding screw nuts(6) as the Figure 5, the hole-plate(1), separate sheath(2), plate(3) and the setting(4) are tightly suppressed together by screws to form M sealing reactive wells. When the reactive wells are not needed, loosen the screws, dismantle the hole-plate and the separate sheath, the probe plate can be used for next step of operation.

Reversible sealing structure is achieved by outside force

The hole-plate(1), separate sheath(2), probe plate(3) and the setting(4) are tightly suppressed together by outside force(8)(e.g. the pressure outside, tongs force) to form M sealing reactive wells as the Figure 6. When the reactive wells are not

needed, remove the outside force, dismantle the hole-plate and the separate sheath, the probe plate can be used for next step of operation.

Reversible sealing structure is achieved by fasteners

The hole-plate(1) has 2 positive fasteners(9), the setting(4) has 2 negative fasteners(10), as the Figure 7. The plate(3) is put on the setting(4) , the hole-plate(1) installed separate sheath(2) is put on the probe plate(3), then the 2 positive fasteners(9) are pressed into the negative fasteners, the hole-plate(1), separate sheath(2), probe plate(3) and the setting(4) are tightly suppressed together to form M sealing reactive wells. When the reactive wells are not needed, the 2 positive fasteners are pressed down and unchained the connection of the 2 negative fasteners, dismantle the hole-plate and the separate sheath, the probe plate can be used for next step of operation.

Reversible sealing structure is achieved by permanent magnet

The permanent magnet(11) has build in the setting(4) as the Figure 8, the hole-plate(1) is made of stainless steel(when the separate sheath is not used, it is made of the combine material of stainless steel covered a layer of rubber), when the plate(3), separate sheath(2) and the hole-plate(1) are put on the setting(4) in file, the permanent magnets(11) has the magnetic suction force to the steel hole-plate(1), then the hole-plate(1), separate sheath(2), probe plate(3) and the setting(4) are tightly suppressed together by the force to form M sealing reactive wells .

Reversible sealing structure is achieved by electromagnet

The electromagnet structure(12) has build in the setting(4) as the Figure 9, the hole-plate(1) is made of stainless steel(when the separate sheath is not used, it is made of the combine material of stainless steel covered a layer of rubber), after the plate(3), separate sheath(2) and the hole-plate(1) are put on the setting(4) in file, the electromagnet structure(12) is electrified and the magnetic suction force to the steel hole-plate(1), then the hole-plate(1), separate sheath(2), probe plate(3) and the setting(4) are tightly suppressed together by the force to form M sealing reactive wells.

The reaction and scanning of the biochip

The probe plates are used those made in Example 1 and the biochip are installed according to Example 2 to Example 8 to achieve 7 different sets. After affinitive reaction and pure reaction, the biochips are disconnected and the separate structures are removed, the probes are scanned.

The 3 negative samples(number 1^-, 2^-, 21^-) and 9 positive samples(number 1^+, 4^+, 7^+, 21^+, 24^+, 31^+, 35^+, 38^+, 39^+) are selected from the standard panel(Chinese National Institute For The Control of Pharmaceutical and Biological Product), the 50ul said samples diluted by 20 times are added into each well of the said 7 sets biochips. After incubated at 37 ℃ for 1 hour, the biochips are washed, 50ul rhodamine labeled anti human IgG-A-M is added into the wells, incubated at 37 ℃ for 1 hour, the wells are washed by wash solution 4 times, distilled water 2times and alcohol absolute, when the biochips are dry, disconnect and the separate structures are removed, the probes are scanned and the data are processed.

Table 1: The test result of different samples using the biochip of Example 4

sample	Result of RIBA	Result of biochip			
		Anti-HCV	Core	NS3	NS4
1^-		545(45)			
2^-		657(63)			
21^-		589(59)			
1^+	$NS3^+$	13254(768)	325(59)	352(49)	348(52)
4^+	$NS3^{++}$	7453(879)	469(56)	5498(420)	505(42)
7^+	$NS3^{++++}$	12432(650)	342(54)	4563(654)	324(29)
21^+	$NS3^{++++}$	28547(3965)	356(41)	13568(2904)	367(41)
24^+	$NS3^{+++}$+$NS4^+$	21784(2509)	395(42)	481(65)	7548(1241)
31^+	$NS3^+$+$NS4^+$	12461(1973)	387(54)	5248(657)	21765(1534)
35^+	$NS3^{++++}$	25423(1795)	476(67)	6830(597)	501(67)
38^+	$NS3^+$	10568(798)	524(45)	8675(805)	596(60)
39^+	$Core^{++}$	39034(2543)	43985(3098)	289(45)	286(67)
blank	5833(49)				

The data of the result of biochip are the statistic average, the data in the parentheses are their standard deviation.

All the results of the 7 sets of the biochip are consistent(the data of the results are omited), and the results of the plane biochip used at present of the biochip are also consistent(the data of the results are omited).

The biochip of this invention can prevent the leakage among the reactive wells, and the structures of reactive surface are in one plane when the biochip is scanning, so the technical difficulty during the multi-sample test is decreased, the test cost is decreased.

Abstract

This invention presents a kind of biochip using reversible connection parts and can test multi-sample on one solid support, the biochip comprises of a setting, a probe-plate on the setting, a hole-plate with the holes correspond to the probe arrays on the probe-plate, localizing pins and reversible sealing structure to seal up the surfaces between the probe plate and the hole-plate to avoid the leakage, the probe plate includes multi-arrays, each array can test multi-items. The liquid medium in the wells will not get over each other when reaction step since the action of the sealing structure, the separate sealing structures are removed before the probe plate is scan, the probes plate can be scanned in a close distance. The advantages of the biochip are the biochip can be installed and dismantled according to the requires of different steps, extends the analysis range and saves the analysis time, and has low cost for analysis of biochip on the base of guarantee the test quality.

Figure Illustration

毛细管生物芯片装置

(Chinese patent publication number 2583395y)

Claims

1, A kind of capillary biochip device, comprises transparent capillary(1) in which there is capillary fibre(thread)(2). There is stable sample line(spot)(3), corresponding marker (4), positive contrast line(5) and negative contrast line(spot)(6) bound on the capillary fibre(2).

2, The device of claim 1, said marker(4), sample line(3), positive contrastline(5) and negative contrast line(6) are arranged orderly from the bottom up. The distances of near two lines of marker(4), sample line(3), positive contrastline(5) and negative contrast line(6) are from 0.01 to 1.0mm and form a region for a testing item.

3, The device of claim 1 or 2, there are over two different regions for testing items on the capillary(1). The distance between two regions is form 0.1 to 2.0mm.

A kind of capillary biochip device

Detailed description

This practical new pattern pertains to life science area, referring to a device, especially a capillary biochip device, used for test, recognition and identification of gene, protein, medicament, tissue or cells.

The present biochip means the high, medial, low densities probe microarray of nucleic acid comprising cDNA, mRNA, PNA and protein (polytide, enzyme molecule, antigen and antibody), medicament, tissue or cells coated on solid support. The mutual biological large molecules are arranged orderly on solid support (glasses, silicon, china) through

the particular specific recognizing abilities, they react or hybridize with samples and corresponding markers simultaneously, then you can get a great deal of useful life science information through auto reading equipment.

Gene chip, also named DNA chip, is a high or medial density probe microarray of nucleic acid(cDNA, mRNA, PNA). Its preparative methods as follows: firstly, using optical etching and in situ synthesis chemical technology, a great deal of nucleic acid probes with specific sequences are solidified orderly on support plate; secondly, the purified nucleic acid probes are spotted (printed) on support plate using auto spotter; thirdly, the gene probe is grown on the end of optical fibre using light-directed technology; finally, the DNA is in situ synthesis on support plate using molecule seal method; the gene arrays are formed which are stored a great deal of useful life information.

Protein chip also named polypeptide biochip, which is made by immobilizing protein (polypeptide, enzyme molecule, antigen and antibody) on solid support, using the principle of protein molecule specific combination with ligand molecule(antibody, antigen and receptor), and using related marker to detect antigen, antibody, receptor, enzyme or polypeptide on a large scale. Its preparative methods as follows: firstly, purified protein molecule is immobilized on the solid support by spotting or printing; secondly, the polypeptide with particular amino acid sequence solidify orderly on the solid plate using light-directed polypeptide synthesis technology.

The usual solid support of biochip comprises: organic and inorganic polymer film, glasses, silicon and china. These supports usually are modified, that is to say, the supports are bound with different active groups using different active reagents through chemical reaction, so the

active groups can be covalent bond with the biological molecules, then form different biological specific affinity solid plate that is biochip, which are used for testing kinds of activated biological molecules such as protein, polytide, enzyme, nucleic acid, antibody and antigen, medicament, receptor, tissues and cells. Some of the solid plate are construct micro structures (micro concave, micro channel, micro trough, micro tube and micro pump), to make for samples processed and agents distribution.

The preparation technics of biochip is very complex and need special expensive equipment. Its means of testing is very rigorous and it is not fit to routine application.

The aim of this practical new pattern is to provide a simple and speedy capillary biochip device in batch, its result is precise and reliable, its result is easily observed and fit for production.

The task of this practical new pattern is accomplished as follows:

A kind of capillary biochip device, comprises transparent capillary(1) in which there is capillary fibre(thread)(2). There is stable sample line(spot)(3), corresponding marker (4), positive contrast line(5) and negative contrast line(spot)(6) bound on the capillary fibre(2).

After adopting said structure of this practical new pattern, the capillary with capillary fibre is used, the aim can be gotten using only one step of test, recognition and identify of gene, antibody, antigen or medicament, especially cells.

The operation of this practical new pattern is simple and speedy, its result is precise and reliable, its result is easily observed. It fits for life science area of diseases related test, recognition and identify including

gene, pathogens, medicament, tissue and cells. When this practical new pattern is used, the indrawn of sample to be tested is only once it can be disposed and related life science information can be gotten.

The enclosed figures are sketch maps of this practical new pattern.

According to the figures, there are further detailed descriptions.
In the enclosed figures: capillary (1), capillary fibre(2), spot line (3), marker (4), positive contrast line (5), negative contrast line (6).

As the figures, the capillary biochip device of this practical new pattern comprises transparent capillary(1) in which there is a capillary fibre(thread)(2). There is stable sample line(spot)(3), corresponding marker (4), positive contrast line(5) and negative contrast line(spot)(6) bound on the capillary fibre(2). The outside is transparent capillary(1), which holds capillary fibre(2) and spreads the sample. Capillary fibre(2) includes stable spotted material line(3). The material is one or their combination of following biological molecules: DNA probe, cDNA probe, PNA probe, mRNA, antigen and antibody (monoclone antibody with different epitopes, human anti-IgM capture antibody, human anti-IgG antibody and secondary antibody), receptor of medicament, lectin, cells or tissue. When over two dots(3) of samples are parallel arrayed from bottom to top, there are over two related markers(4) on capillary fibre(2). The markers are any one or their combination of gold marker, iron marker, enzyme marker, fluorescence marker, chemiluminescence marker, dyestuff marker and radioactive marker. Moreover, there are also over two positive contrast(5) and negative contrast(6) in capillary fibre(2). In the region for the same testing item, the related markers(4), dots(3), contrast(5) and negative contrast(6) for the same item are arranged from bottom to top, their space is 0.01-1.0mm. The space between the testing regions for different items is

0.1-2.0mm, so good for distinguish different testing regions and test. The testing regions for different items are arrayed in equidistance on capillary fibre(2) form bottom to top. The enlargement of capillary causes the coloration of line or spot more clearly and recognizable.

The underside of said device is insert into the sample, the indrawn sample into transparent capillary by capillarity of the capillary and fibre, the sample can move upwards along transparent capillary vessel and soak capillary fibre rapidly, as the same time, the target in the sample reacts or hybridizes with the material of the dot and the marker. After 4-60 min in room temperature, the result is observed or read by equipment after wash, the result of test is identified according to the coloration line. If there is no coloration line in testing region of one item, the sample is negative, on the contrary, the sample is positive. For positive sample, it can also compare with the positive contrast line for half quantitative test according to the depth of the color of coloration line. Furthermore, if the positive contrast line shows color, the device is available, otherwise the device is unavailable.

数据查询

数据库：药品注册批准信息	
药品通用名	丙型肝炎病毒分片段抗体检测试剂盒(蛋白芯片)
英文名	proteinchip dianostic kit for antibodies to hepatitis c virus
申请分类	新药
批准日期	2002 年 10 月 16 日
申请限制	-----
限制到期日	-----
备注	本数据查询提供最近批准注册各类药品的基本情况，用于向申报同品种的申请人提供参考信息。因来源关系，数据中可能存在问题。请仔细比较同品种信息，如发现问题，请致电010-88373856，或将问题页面打印，传真至 68362745，信息组收。

Data-base: the Information for Registration and Warrant of Physic	
Physic name	proteinchip dianostic kit for antibodies to hepatitis c virus
Application sort	new
Permit date	16 October,2002
Application limit	----
Maturity of limit	----
remark	Ir provide the basal Information for Registration and Warrant of Physic
